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The Effect of Enamel Matrix Derivative/Papilla Reflection Surgery on the Clinical and Alveolar Bone Outcomes in Periodontal Maintenance Patients

Erica E. Jasa
University of Nebraska Medical Center

THE EFFECT OF ENAMEL MATRIX DERVIATIVE/PAPILLA REFLECTION SURGERY ON THE CLINICAL AND ALVEOLAR BONE OUTCOMES IN PERIODONTAL MAINTENANCE PATIENTS

by

Erica E. Jasa, D.D.S.

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Advisory Committee:

Amy C. Killeen, D.D.S., M.S.

Jeffrey B. Payne, D.D.S., M.S.

James K. Wahl III, Ph.D.

Sung K. Kim, D.D.S.

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Erica E. Jasa, D.D.S., M.S.

University of Nebraska, 2019

Advisor: Richard A. Reinhardt, D.D.S., Ph.D.

The purpose of this study was to determine if local application of enamel matrix derivative (Emdogain; EMD), combined with minimally invasive papilla reflection/root preparation (PR/RP), is effective in improving probe depth (PD), clinical attachment level (CAL), and interproximal bone height (IBH) in persistent 6-9 mm periodontal pockets in patients on periodontal maintenance therapy (PMT). Fifty periodontal maintenance patients with advanced chronic periodontitis presenting with a 6-9 mm interproximal PD were included in study. Experimental (PR/RP+EMD; n=24) and control (PR/RP+S; n=26) therapies were randomly allocated. Roots were treated with reflection of interproximal papillae, root planing assisted with endoscope evaluation, and acid etching, followed by EMD or saline application. Clinical measurements were collected at baseline, six months, and 12 months post-therapy. IBH measurements were made on standardized vertical bitewing radiographs taken at baseline and 12 months. Both PR/RP+EMD and PR/RP+S resulted in significant improvements in clinical outcomes (PD: -2.3 $\pm$ 0.2 mm, -2.4 $\pm$ 0.2 mm, $p<0.0001$; CAL: -1.8 $\pm$ 0.3 mm, -2.2 $\pm$ 0.3mm, p,0.0001) and stable IBH (-0.2 $\pm$ 0.18 mm, -0.33 $\pm$ 0.18 mm, $p>0.05$), from baseline to 12 months. No significant differences were found in clinical outcomes between the experimental group and the control. The addition of EMD to PR/RP does not significantly improve clinical outcomes compared to PR/RP alone in periodontal maintenance patients.

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LIST OF ABBREVIATIONS

AAP	American Academy of Periodontology
BOP	bleeding on probing
CAL	clinical attachment level
CEJ	cementoenamel junction
EMD	enamel matrix derivative (Emdogain)
EDTA	ethylenediaminetetraacetic acid
GCF	gingival crevicular fluid
GTR	guided tissue regeneration
IBH	interproximal bone height
MIST	minimally invasive surgical technique
MWF	Modified Widman Flap
OFD	open flap debridement
OHI	oral hygiene instructions
PD	probing depth
PDL	periodontal ligament
PL	plaque
PID	positioning indicating device
PMT	periodontal maintenance therapy
PR/RP+EMD	papilla reflection with root planing and emdogain application (test therapy)
PR/RP+S	papilla reflection with root planing and saline application (control therapy)
REC	gingival recession
SRP	scaling and root planing

CHAPTER 1: INTRODUCTION

Periodontitis is an inflammatory disease which affects the hard and soft tissue attachment and supporting structures of the dentition (AAP Parameters of Care, 2000), affecting approximately 42% of the population in adults 30 years of age or older in the United States (Eke et al., 2018). The prevalence and severity of periodontitis increases with age and has a higher occurrence rate in males (Eke et al., 2015).

Diagnosis of periodontitis is made based on clinical parameters including: presence or absence of bleeding on probing (BOP), severity of bone loss and attachment loss, periodontal pocketing, extent and pattern of teeth involved, medical and dental risk factors, pain, ulceration, and amount of plaque (PL) and calculus present (AAP Position Paper, 2003). The type and severity of periodontitis (chronic vs. aggressive, localized vs. generalized, or periodontitis as a manifestation of systemic disease) must be accurately differentiated from other similar diagnoses to allow for and ensure proper treatment protocol (Armitage, 1999).

Treatment of periodontitis via the removal of bacterial biofilm, calculus, and toxic cementum through scaling and root planing (SRP) is considered the “gold standard” (Cobb, 2002) and has been successful in showing improvements in clinical parameters and a reduction of inflammation (Kaldahl et al., 1996a, Becker et al., 2001). Periodontal pockets that continue to have BOP and/or an increasing probing depth (PD) are indicative of continued inflammation (Amato et al., 1986), and the presence of bacteria (Wilson et al., 2008), and are at risk of further breakdown and disease progression (Renvert et al., 2002). These non-responding sites may benefit from surgical treatment or additional rounds of SRP (Kaldahl et al., 1996b).

Once a patient has been determined to be stable, the patient is placed into a periodontal maintenance therapy (PMT) program consisting of dental visits every three-four months where he/she receives continued periodontal evaluation and monitoring, biofilm and root surface decontamination, and oral hygiene instructions (OHI). Participation in PMT is critical to the long-term success of periodontal treatment (Nyman et al.. 1975; Nyman et al., 1977; Becker et al,. 1984a; Becker et al., 1984b; Wilson et al., 1987).

When treating periodontitis during PMT, not all patients remain stable with conventional treatment modalities. For instance, isolated deep interproximal pockets (6-9 mm) commonly develop. Therefore, adjunctive therapies have been developed and added to traditional periodontal therapies. These adjuncts include systemic antibiotics (Sgolastra et al., 2014), local delivery of antibiotics (Kinane & Radvar, 1999), subgingival irrigation (Jolkovshy et al., 1990), lasers (Cobb, 2006), and varying biologics and growth factors (Mobelli, 2005). These adjunctive therapies aim to decrease the bacterial load, aid in reduction of inflammation, and stimulate new attachment to the root or bone growth. Outcomes to these approaches during PMT are rarely reported and are often suboptimal (AAP, 2006). Additionally, conventional periodontal surgery carries morbidity that is often unacceptable to the patient. Less invasive approaches with local application of drugs known to stimulate periodontal regeneration would add a valuable option for retreatment.

The use of enamel matrix derivative, or Emdogain (EMD), as an adjunct to periodontal therapy is proposed for regeneration of lost periodontal structures (Hammarstrom et al., 1997; Sculean et al., 1999). The use of EMD has most often been studied in conjunction with surgical periodontal treatment and shows varying degrees of success and efficacy with its use (Heijl et

al., 1997; Froum et al., 2001, Sculean et al., 2001; Sanz et al., 2004). Although little impact was noted when adding sulcular EMD following SRP (Gutierrez et al., 2003; Mombelli et al., 2005), simple papilla reflection has not been tested to allow enhanced root preparation and EMD application during PMT. Much of the data surrounding the use of EMD are conflicting or has been funded by the company which produces EMD. Evidence of the use of EMD in periodontal maintenance patients is lacking, and, therefore, further research is indicated in the use of EMD in this patient population.

The hypothesis of the current study is that interproximal papilla reflection, root preparation (root planing) with fiberoptic visualization and etching, with or without EMD application as the variable, will reduce probing depth, clinical attachment loss, and improve bone height of deep PMT pockets.

CHAPTER 2: LITERATURE REVIEW: PERIODONTITIS

Periodontitis is an inflammatory disease affecting the supporting structures of the dentition. Periodontitis presents as inflammation of the gingiva and periodontal ligament (PDL), causing damage to alveolar bone and cementum. The disease can clinically manifest itself as gingival erythema with an increase in PD, loss of clinical attachment level (CAL), tooth mobility, BOP, suppuration, abscess formation, gingival recession (REC), and discomfort. If left untreated, it may result in further loss of attachment and eventual tooth loss (AAP Position Paper, 1999).

Periodontitis is a complex and multifactorial disease process consisting of interactions between pathogens and the host's response to them (Preshaw, 2008). The main etiologic cause of periodontal disease has been attributed to bacterial plaque and calculus (Socransky & Haffajee, 1992). However, bacteria alone may not be enough to account for the progression of the disease. Host susceptibility has been found to be a vital part of the disease progression. Over 500 microorganisms have been identified in periodontal disease, but it is thought that only 10-20 of these may play a part in the pathologic etiology of the disease (Moore, WE. & Moore, LV., 1994). Many factors may allow for a progression of disease. Periodontitis may occur when the pathogen is of a virulent type and possesses genetic factors to initiate disease, the host is susceptible to the pathogen, the pathogen is present in number sufficient to exceed the threshold of the host, other bacterial species do not alter the progress of the disease, and the local environment is conducive to the pathogens' virulence properties (Socransky & Haffajee, 1992). Certain genetic factors have also been suggested as contributors to various forms of periodontitis, including neutrophil dysfunction (Van Dyke et al,. 1985) and IL-1 polymorphism (Kornman et al., 1997).

The goal of treating periodontal disease is to halt the progression of periodontal destruction by reducing inflammation and restoring the patient to a comfortable and functional dentition (Zander et al., 1976). Treatment can be achieved by non-surgical therapy, surgical therapy, or a combination of both. These treatments allow for a disruption of the bacteria, detoxification of the root surface, and a reduction in the microbial load, thereby reducing inflammation in the pocket and allowing for the host to compensate. Clinical improvement is measured using change in PD, presence of BOP, plaque index, and gain in CAL (Haffajee et al., 1997).

Non-surgical periodontal therapy, or SRP, has been coined the "gold standard" of periodontal treatment (Cobb, 2002). This consists of the removal of bacterial plaque and calculus by instrumentation of the crown and root with hand instruments and/or ultrasonic instruments. Removal of the plaque and calculus results in a reduction of the bacterial load (Socransky et al., 2013) and detoxification of the root (Nishimine & O'Leary, 1979). Many studies have demonstrated improvements in periodontal health and clinical parameters following SRP (Kaldahl et al., 1996a; Becker et al,. 2001; Mialoa et al., 2015).

If signs of disease activity, such as an increase in PD, BOP, and continued attachment loss, persist following mechanical therapy, other therapies including surgical intervention, and/or the use of adjunctive pharmacotherapeutic and biomaterials may be considered. Additional risk factors which may be contributing to the disease process also need to be identified such as occlusal trauma, iatrogenic restorations, malocclusion and crowding, and smoking. Continued treatment of non-responding sites is crucial as sites which have

residual signs of inflammation and deep PD are more likely to progress and experience recurrence of disease (Claffey et al., 1990; Renvert & Persson, 2002; Matuliene et al., 2010).

Once a patient's periodontal status is determined to be stable, he/she will enter into periodontal maintenance and will be monitored for changes in the periodontium during more frequent dental visits and cleanings, usually every three-four months. The goals of PMT are: 1) to prevent or minimize the recurrence of disease progression; 2) prevention or reduction of the incidence of tooth loss; and 3) to increase the probability of locating and treating other conditions or diseases found within the oral cavity in a timely manner (Cohen, 2003). PMT helps to maintain periodontal health by decreasing etiology and stabilizing the attachment. At each PMT visit, an evaluation of the patient's periodontal status is performed by the collection of clinical measurements. Removal of supragingival and subgingival bacterial plaque and calculus is then performed, along with a review and reinforcement of oral hygiene instructions, and a determination of any additional needed treatment (Cohen, 2003).

Studies have shown that patients undergoing regular PMT have less incidence of periodontal breakdown and keep their teeth longer than those who are erratic or non-compliers (Wilson, 1987). Regular maintenance therapy along with proper oral hygiene may resolve gingivitis and help prevent loss of periodontal tissue support (Axelsson et al., 1991). Patients who receive periodontal therapy and do not follow through with maintenance, do not see long term improvements in PD and bone levels. In these patients, the improvements seen following periodontal therapy are ultimately lost, and they eventually return to a diseased state (Becker, 1984a; Becker, 1984b; Alexelsson & Lindhe, 1981). Therefore, participation in a periodontal maintenance program is crucial to the long-term success of periodontal therapy and the stability

of the periodontal tissues (Nyman et al., 1975, Ramfjord, 1982, Becker et al., 1984a, Becker et al., 1984b).

CHAPTER 3: LITERATURE REVIEW: ENDOSCOPE AND MINI-FLAP

The primary objective of periodontal treatment is to remove the plaque biofilm, calculus, and contaminated root surface, thereby lowering the threshold of bacteria (Cobb, 1996). However, restricted access in deep pockets can create challenges making non-surgical treatment difficult. Studies have shown that there is a threshold of severity at which instrumentation becomes less effective. Curette efficacy ranges from 2-4 mm, with calculus free surfaces only being found up to 3.73 mm (Stambaugh, 1981). As the pocket depth increases, there is a higher correlation of percent of residual calculus present (Rabbani et al., 1981).

The detection of subgingival calculus is not a precise action. Calculus detection is most often done with the light tactile touch of an explorer, relying on the clinician's opinion on whether or not calculus is present. This can also lead to inconsistencies between clinicians, as there are differences in what a clinician determines a smooth root surface to be. Studies evaluating the accuracy of subgingival calculus detection have shown 75% accuracy in the clinician's ability to determine the presence of calculus, and a 50% accuracy in the clinician's ability to determine the surface free of calculus (Sherman et al., 1990). The microscopic presence of calculus was always found to be greater than the clinical detection (Sherman et al., 1990).

The effectiveness of instrumentation can also be affected by tooth anatomy or position in the arch. Interproximal surfaces were found to have residual calculus more frequently than the facial and lingual surfaces, both microscopically and clinically (Sherman et al., 1990). Molars have been shown to have more residual calculus than non-molar sites (Sherman et al., 1990). The morphology of furcations are complex, making them more difficult to clean, and root

concavities add to the complexity (Fleischer et al., 1989). In deeper pockets, in order to ensure more calculus removal and better access to the depth of the pocket, adjunct procedures may need to be combined with non-surgical therapy.

Endoscope

The precise detection of subgingival calculus and the evaluation of the root surface and soft tissue is critical for diagnosing and treatment planning during periodontal therapy, if successful outcomes are desired (Stambaugh et al., 2002). The use of a dental endoscope can aid in subgingival visualization of the root surface and calculus detection. The dental endoscope uses a thin fiber-optic cord inserted into a sheath with a light source to illuminate the root surface. The sheath also provides constant water flow to clear the visual field of biofilm, calculus, blood, and other debris. The images are magnified and transmitted back to a display screen for visualization in real time. The endoscope can be used in conjunction with ultrasonic and hand instruments, which may allow the clinician to achieve better clinical results. In a systematic review, it was determined that the use of a periodontal endoscope may provide an additional benefit of calculus removal compared to SRP alone, but no significant differences were found in clinical parameters with respect to BOP, gingival index, and PD with the aid of the endoscope compared to SRP alone (Kuang et al., 2017). The percentages of residual calculus present after periodontal therapy performed with the aid of the endoscope were significantly less than the amount present following SRP; however, SRP with the endoscope required significantly more time for debridement than SRP alone (Kuang et al., 2017). Some studies reported greater reductions in gingival index and BOP with the use of the endoscope (Wilson et al., 2008; Blue et al., 2013). The endoscope provides a minimally invasive technique to aid

clinicians in the ability to visualize periodontal conditions, and, therefore, may improve calculus detection (Stambaugh et al., 2002).

Minimally Invasive Surgical Techniques

In deep periodontal pockets which do not respond to non-surgical therapy, surgical access has been shown to aid in calculus removal, especially in furcation areas or deep pockets where closed instrumentation is difficult (Fleischer et al., 1989). However, periodontal surgery increases morbidity, chances for infection, and postoperative pain for the patient. Minimally invasive non-surgical periodontal therapy provides another alternative to traditional surgical periodontal therapy, in order to achieve subgingival debridement with minimal tissue trauma. Minimally invasive procedures consist of localized papilla reflection to allow access to an area of localized periodontal destruction and usually involve the use of magnification with either loupes or microscopes. Minimally invasive procedures look to minimize patient postoperative discomfort and maximize the healing potential (Cortellini & Tonetti, 2007). Microsurgery can result in limited soft tissue damage, limited recession, gains in attachment, improvement of soft tissue healing, and a high probability of primary closure (Cortellini et al., 2001). Improvements can also be seen in the rate of healing, reduction of postoperative pain, and improved retention of soft tissue height and contour (Harrel, 1999). Minimally invasive procedures may promote an enhanced clinical improvement in intrabony defects, including larger reductions in probe depths, and gain in clinical attachment level and radiographic bone height compared to non-surgical therapy (Nibali et al., 2015). Sites with interdental papilla reflection also exhibited less residual calculus compared to sites without papilla reflection (Reinhardt et al., 1985). Surgical chair time was also decreased when utilizing

minimally invasive techniques compared to larger surgical flaps (Cortellini & Tonetti, 2009). Improvements seen following a minimally invasive approach appear to heal similarly to single flap surgeries (Trombelli et al., 2007) and appear to be stable long term (Nibali et al., 2018).

Combination of the minimally invasive approach with visualization provided by the periodontal endoscope may allow for enhanced clinical improvements in localized areas of periodontal inflammation. The combination of these two procedures allows the clinician to gain better access in deep periodontal pockets resulting in more calculus removal, while minimizing trauma and maximizing the healing potential for the patient.

When local application of periodontal regenerative drugs and root etching are proposed (as in the current study), access to the root and tissues on the bone surface are necessary.

CHAPTER 4: LITERATURE REVIEW: EMDOGAIN

The aim of periodontal treatment is to halt the progression of periodontal attachment loss and establish a dentition that is functional and comfortable for the patient (Zander et al., 1976). Periodontal treatment may also consist of procedures designed to regenerate lost periodontal tissues (Philstrom & Ammons, 1997). Many regenerative procedures have been investigated and have shown success, including root conditioning, grafting with various materials (autografts, allografts, xenografts), and the use of barrier membranes. More recently, the use of biologics and growth factors have been added to the repertoire to aide with periodontal regeneration procedures. One which has shown particular success is enamel matrix derivative, or Emodgain (EMD). EMD is an extract of embryonic enamel matrix from six month old piglets, and it is thought to induce mesenchymal cells to mimic the process of the development of the root and periodontal tissues (Venzia et al., 2004). EMD is mostly composed of amelogenins which are involved in the formation of enamel and development of acellular cementum (Brookes et al., 1995). Cementum deposition aides in the development of the periodontal attachment apparatus and is required for the formation of both the periodontal ligament and alveolar bone (Armitage, 1991). Deposition of enamel matrix proteins onto a dentin surface initiates the cementogenesis process. Once cementum has been laid down onto an enamel-matrix covered dentin surface, the attachment apparatus can develop (Slavkin et al., 1989; Lindskog & Hammarström et al., 1982).

EMD appears to enhance proliferation of PDL cells, but not epithelial cells, thus allowing for regeneration of the periodontal tissues (Gestrelius et al., 1997). EMD has also been shown to significantly increase attachment rate, growth factor production (TGF-β1; PDGF-AB),

proliferation, and metabolism of human PDL cells (Lyngstadaas et al., 2001). EMD adsorbs to hydroxyapatite and collagen on denuded roots forming insoluble complexes which can remain detectable for up to two weeks. This time frame appears to be sufficient to allow for recolonization of the periodontal ligament cells (Gestrelius et al., 1997). In vitro studies have demonstrated EMD's ability to limit epithelial downgrowth on the root surface, which may be similar to that seen by the mechanical prevention achieved with barrier membranes (Hammarström, 1997; Venzia et al., 2004).

Surgical Periodontal Therapy + EMD

Clinical trials evaluating the use of EMD have most often been done evaluating EMD in conjunction with surgical procedures in intrabony periodontal defects. In a split mouth study, EMD as an adjunct to Modified Widman Flap (MWF) surgery for treatment of intrabony defects, showed a greater gain in CAL, reduction in PD, and increased gain of radiographic bone fill (66%), compared to MWF plus a placebo (Heijl et al., 1997). Additional studies compared the use of EMD to a placebo, with open-flap debridement (OFD), and found similar results in clinical and radiographic outcomes between the two (Zetterstrom et al., 1997; Pontoriero et al., 1999). A meta-analysis reviewed the studies comparing EMD to OFD and found the EMD groups had significantly higher reductions in PD (4.82 ± 0.02 mm vs. 2.59 ± 0.06 mm, $p = 0.000$) and gains CAL (4.07 ± 0.03 mm vs. 2.55 ± 0.04 mm, $p = 0.000$); no significant differences were seen in the initial PD and CAL between groups (Venzia et al., 2004). In contrast, one clinical trial failed to demonstrate an advantage with the use of EMD in infrabony defects during OFD compared to OFD alone (Rösing et al., 2005).

EMD vs GTR

The gold standard of regenerative procedures is guided tissue regeneration (GTR) with the use of barrier membranes. Studies comparing the regenerative effects of EMD to those of GTR have shown promising results in EMD's regenerative capabilities. The studies show both the use of EMD and GTR lead to comparable improvements in regards to CAL gains and osseous defect fill compared to OFD alone (Kalpids & Ruben, 2003). No significant differences were seen in probing depth reduction between EMD and GTR, with both groups showing an improvement from baseline (Sculean et al., 1999; Pontoriero et al., 1999; Minabe et al., 2002; Venzia et al., 2004). However, GTR has been shown to have more surgical complications compared to EMD, mainly due to membrane exposure, illustrating EMD's clinical advantage in sites where membrane placement may be difficult (Sanz et al., 2004).

Non-surgical Periodontal Therapy + EMD

Clinical outcomes following non-surgical therapy with the addition of EMD have not been studied as extensively. Only two clinical studies have been done evaluating these results. Both studies failed to show any clinical benefits to the use of EMD as an adjunct to non-surgical treatment (Gutierrez et al., 2003; Mombelli et al., 2005). This may be because horizontal defects are less likely to exhibit success following regenerative procedures (WIkesjo & Selvig, 1999) and EMD was not allowed contact with deeper connective tissue and bone. It is, therefore, recommended that EMD be used in conjunction with surgical therapy to fully reap the benefits of its regenerative properties.

EMD + Minimally Invasive Surgical Techniques

The aim of minimally invasive surgical techniques (MIST) is to reduce the postoperative morbidity associated surgical procedures and allow for improved healing. Few studies have investigated the use of EMD with MIST. In studies utilizing MIST with the addition of EMD, improvements were seen in CAL, PD, initial would stability, and defect resolution with limited patient morbidity (Cortellini & Tonetti, 2007; Cortellini & Tonetti, 2009). In another controlled clinical study, the use of EMD did not provide superior benefits on the outcome of the MIST procedure, as both groups reported significant PD reductions, CAL gains, and radiographic bone fill at three and six months (Ribeiro et al., 2011).

Conflicting and limited data on the use of EMD for the treatment of periodontal defects illustrates the need for future, well-controlled, randomized, long-term clinical trials.

MATERIALS AND METHODS

Study Population and Research Design

This 12 month, randomized, double-masked, parallel interventional, clinical trial included randomization of 50 individuals (26 males, 24 females) who were receiving periodontal maintenance therapy at the University of Nebraska Medical Center (UNMC) College of Dentistry. The study protocol flow chart is presented in Figure 1. Inclusion criteria were as follows: 1) 40-85 years old; 2) a diagnosis of advanced chronic periodontitis (majority of patients were Stage III, Grade B; AAP, 2018); 3) with at least one 6-9 mm interproximal periodontal probing depth with a history of bleeding on probing; 4) overall good systemic health; 5) history of regular periodontal maintenance therapy; and 6) willingness to sign consent form. Exclusion criteria were as follows: 1) systemic diseases which significantly impact periodontal inflammation and bone turnover (e.g., chronic use of steroids or non-steroidal anti-inflammatory drugs (>325 mg/d), estrogens, bisphosphonates, calcitonin, methotrexate, antibiotics); 2) surgical periodontal therapy within the past year; 3) interproximal defects with vertical component of >3 mm; 4) pregnant or breast-feeding females. The protocol was approved by the UNMC Institutional Review Board, Omaha, Nebraska (protocol #783-16-FB) and was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013. Recruitment for the study took place from September 2016 to April 2017. The study was conducted from February 2017 to July 2018 and was registered with ClinicalTrials.gov as NCT02972788.

Patient Selection

UNMC periodontal maintenance patients were screened, and those meeting the inclusion criteria were invited to participate and given written informed consent. Randomization was stratified by gender and smoking status, via a preset randomization table. The experimental site of the individuals was assigned from screening data (6-9 mm periodontal probe depth with history of BOP). The test group received papilla reflection and root planning (PR/RP), with visual augmentation using an endoscope, and root etching with ethylenediaminetetraacetic acid (EDTA) for two minutes, and Emdogain (PR/RP + EMD) injected into the open interproximal site, whereas the control group received PR/RP, and root etching with saline (PR/RP + S), in one experimental site per individual.

Sample Collection and Clinical Measurements

One of three calibrated examiners (AK, RR, JP), without knowledge of experimental group assignment, isolated the experimental site with cotton rolls. Supragingival plaque (PL) was removed (and recorded) with curettes, and the site was gently dried with an air syringe. A periodontal paper strip (Periopaper, Proflow, Amityville, NY) was inserted into the experimental interproximal site sulcus on both buccal and lingual until mild resistance was felt, and was left for 30 seconds to collect gingival crevicular fluid (GCF). Next, gingival recession and probing depths were recorded with a UNC 15 probe on the experimental tooth and adjacent tooth at mesial-facial, mid-facial, distal-facial, mesial-lingual, mid-lingual, and distal-lingual by the same calibrated examiner. Clinical attachment level (CAL), was calculated as the sum of REC and PD measurements.

Interproximal bone height (IBH) measurements were made on vertical bitewings made with a modified positioning indicating device (PID) aligner (Figure 2). Measurements were made from the cementoenamel junction (CEJ) to the most coronal aspect of the alveolar crest, where the periodontal ligament space was uniform. If a restoration was present on the tooth surface being measured, measurements were taken from the apical margin of the restoration to the most coronal aspect of the alveolar crest. IBH measurements were made at the treatment site, as well as the interproximal of the adjacent tooth (Figure 3). Interproximal vertical defects of >3mm were used an exclusion criteria for study participation.

Treatment

One of two periodontal residents (EJ, JG), performed the experimental site treatment. A baseline vertical bitewing radiograph was made with a modified PID aligner to standardize radiography beam geometry. Local anesthetic was administered to the experimental site. Surgical reflection of the buccal and lingual/palatal papillae, localized to the experimental site, was done using #12b blade and Molt #2 elevator. Granulation tissue and remaining interproximal soft tissue was removed using scalers (Hu-Friedy, Chicago, IL) and an ultrasonic scaling system (Cavitron, Dentsply, York, PA) was used to allow access to the root and visualization to within 2 mm of the bone. Scaling and root planing of experimental and adjacent interproximal teeth were performed to remove supra- and subgingival bacterial plaque and calculus. Circumferential cleaning was done to prevent any disturbances to the re-approximated papilla during the subsequent periodontal maintenance appointment. Verification of calculus-free and smooth root surfaces was performed using an 11/12 explorer and fiber

optic visualization with an endoscope (Perioscope, Zest Dental Solutions, San Ramon, CA). If the root surface was not adequately prepared, then scaling and root planing was repeated and verified until satisfactory. The root surface and papilla were irrigated with sterile saline to remove any debris from the pocket. The root surface was etched for two minutes with EDTA (Pref-Gel, Straumann, Andover, MA), to further detoxify the root surface, followed by irrigation with sterile saline. Depending on randomization, either 0.3 ml hydrophobic enamel matrix protein (Emdogain, Straumann, Andover, MA) or 0.3 ml sterile saline was placed to the bone and PDL, and deposited up the root surface of the experimental and adjacent interproximal tooth. Any excess was removed and using a damp gauze and compression. The buccal and lingual/palatal papilla were re-approximated. Damp gauze pressure was applied for three-five minutes followed by application of intraoral cyanoacrylate (Periacryl, GluStitch Inc., Delta, BC, Canada) to stabilize clot formation and secure the papilla. Routine periodontal maintenance, including full-mouth debridement and root planing of inflamed pockets (excluding experimental site), was then performed by a registered dental hygienist (MC). Patients were instructed to avoid brushing and interproximal cleaning of the experimental site for six weeks, per the Straumann Emdogain protocol. Patients were provided a bottle of Listerine mouthrinse (Johnson & Johnson, New Brunswick, NJ), to rinse with two times per day for the six week postoperative period.

Patients were asked to return for postoperative visits at two and six weeks along with periodontal maintenance recalls at three, six, nine, and 12 months. GCF collection was repeated at two weeks, six months, and 12 months. The same measurements completed at baseline were recorded at six and 12 months. One dental hygienist completed all the periodontal

maintenance therapies. Participants were questioned about adverse events at two weeks, six months, and 12 months. See clinical photos of the procedure in Figure 4.

Figure 1: Flow of Study Design

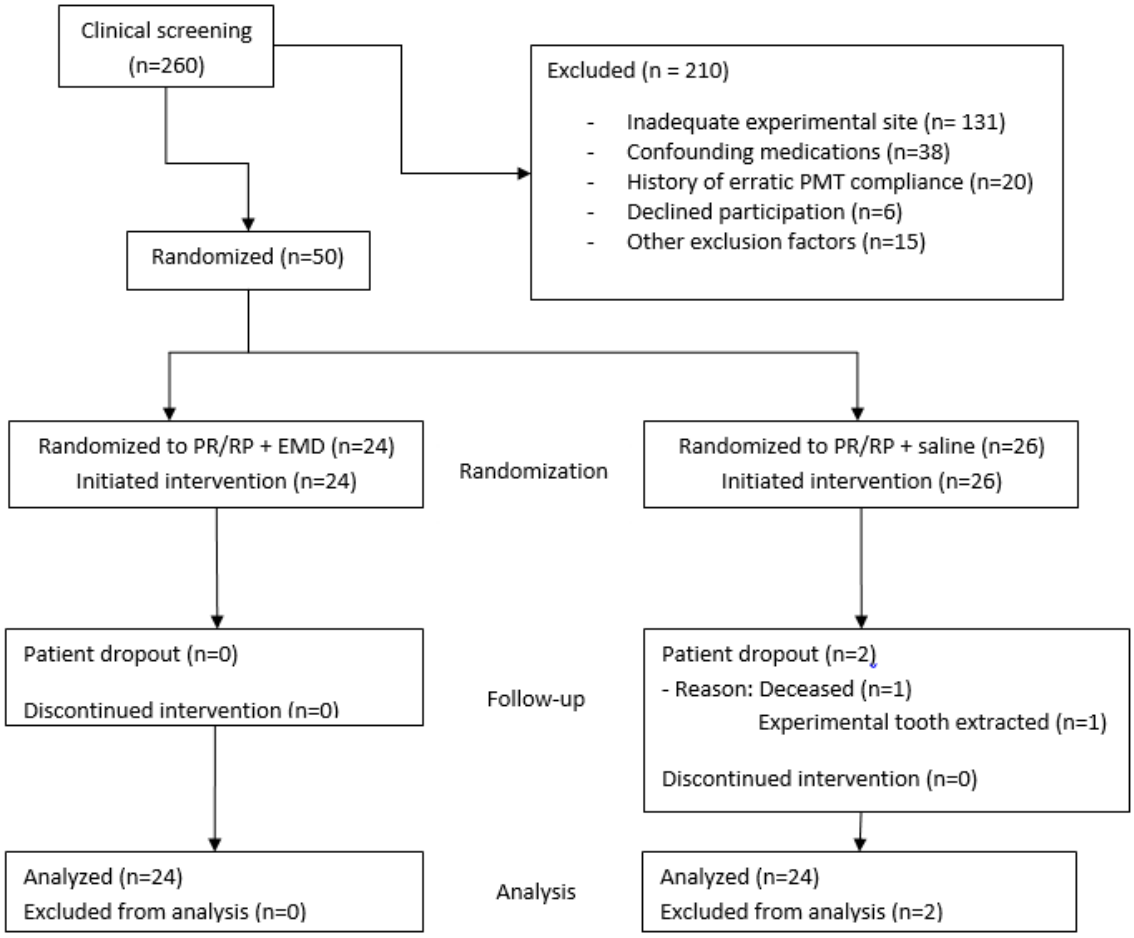
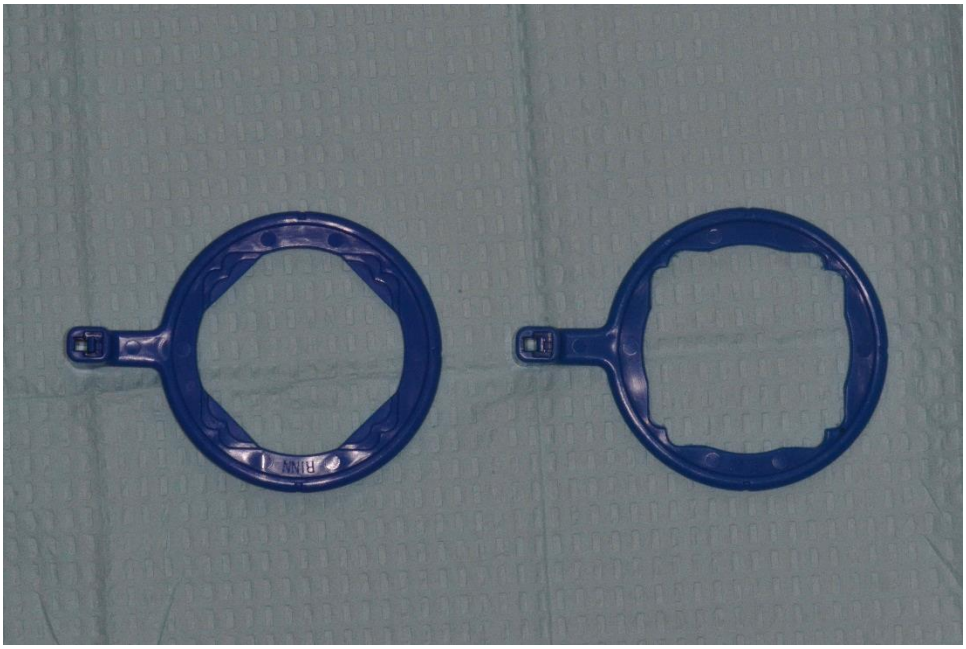
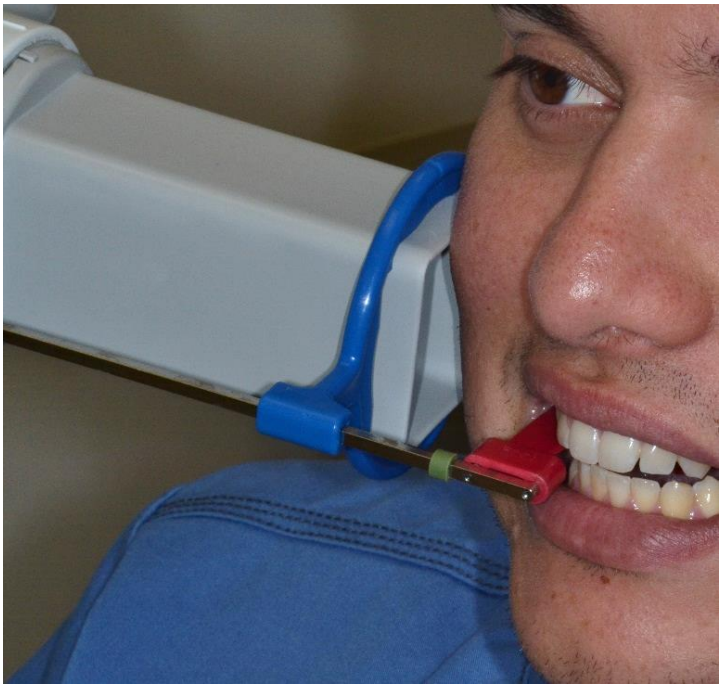


Figure 2: Radiographic Modified PID Aligner



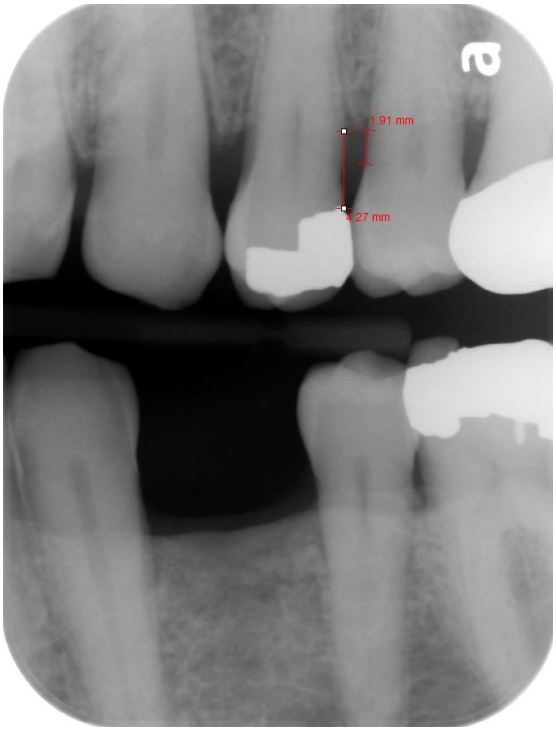
Left: Non-modified PID aligner Right: Modified PID aligner (used in this study)



Modified PID aligner in use

Figure 3: Radiographic Bone Height Measurements

Initial Radiograph



12 month Post-Op Radiograph

Figure 4: Clinical Photos of Surgical Procedure

Baseline PD Measurement

Root Planing with Endoscope

EMD Application

Papilla Reflection Closure

12 Month Post-op

Statistical analyses:

For patients to be enrolled in this study, they must have had a probing depth of at least 6 mm on either the buccal or lingual of the interproximal site. For purposes of analyses for PD and CAL, only the measurement from the site with the deepest probing depth on the treatment tooth was analyzed, if both sides had equally deep probing depths, then treatment site measurements were averaged across the buccal and lingual.

For IBH and treatment site PL measurements, both buccal and lingual sites were assessed, regardless of the site of deepest probing depth. PL was considered present at baseline if at least one site (buccal or lingual) had the condition present. Differences in the proportion of patients with PL at baseline vs. twelve months was assessed using McNemar's tests, separately for Emdogain and saline patients.

Means were computed across buccal and lingual sites for each measurement. For each variable, a new variable indicating change was calculated by subtracting the baseline values from the 12 month values. Differences between treatment (EMD or saline) at baseline, 12 months, or the change from baseline to 12 months were assessed using Wilcoxon Rank Sum test or T-test. Differences in measurements within groups over time, were assessed using Signed Rank tests. When the initial measurement was significantly associated with a change in measurement, model adjusted change in measurement means were calculated for three different values of initial measurement: the 10th percentile, mean, and 90^{th} percentile. Significant main effects of variables with more than two levels were further assessed using post-hoc pairwise comparisons which used simulation to adjust p-values for multiple

comparison. P-values less than 0.05 were considered to be statistically significant. All analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC).

To determine the reliability of interproximal bone height measurements, replicate measurements were made for five patients (40 sites), by the same rater (EJ); replicates were measured for two areas (treatment site and adjacent site), at baseline and final time points. Replicate measurements were made 1 week apart. Single measure intraclass correlations (ICCs) for absolute agreement were calculated using two-way mixed effects models for each of the areas, using SPSS software, version 23 (IBM Corp., Armonk, NY).

RESULTS

Examiner calibration:

The data collection examiners (AK, JP, RR) were calibrated for inter-examiner reliability and reproducibility using 48 randomly chosen sites. PD and CAL was reproducible at ± 1 mm for least 85% of sites (AK-RR = 88, 92%; AK-JP = 92, 90%; RR-JP = 94, 96%). Intra-examiner reliability and reproducibility for interproximal bone height measurements (collected by EJ) were performed using 40 sites. Intraclass correlations were used for a two-way mixed model assessing absolute agreement - single measurement (ICC = 0.95325).

Patient characteristics:

All 50 patients who were eligible and invited to participate in the study agreed to do so. Two patients were unable to complete the study (4% dropout rate). One patient passed away before the six month periodontal maintenance appointment. The second patient was eliminated from the study due to extraction of the experimental tooth before the six month periodontal maintenance appointment. Both reasons for patient dropout were not believed to be related to any dental therapy provided during the study. Forty-eight patients completed the study. All study patients were asked at two weeks, six weeks, three months, six months, nine months, and 12 months post-therapy if any symptoms or problems were experienced. Very few postoperative complications were encountered or reported throughout the study. At two weeks, seven patients reported sensitivity to cold, three reported increased food impaction at the treatment site, and four reported slight pain requiring them to take acetominophen for two days. By 12 months postoperatively, only two patients reported sensitivity to cold. The mean

age of the patients was 67 (EMD) and 65 (Saline); there was no significant difference between ages of the patients in the two groups (p=0.41). Patient characteristics at the baseline examination are displayed in Table 1.

Clinical outcomes:

The mean baseline and 12 month post-treatment results for respective changes in clinical outcomes between groups over time are reported in Tables 2-7. As outlined in Table 2, no differences were found in PD (p=0.14) or CAL (p=0.58), at baseline between groups. Both the PR/RP+EMD and PR/RP+S groups saw a reduction in PD (-2.29 ± 0.21 mm, p<0.0001; -2.39 ± 0.21 mm, p<.0001) and gain in CAL (1.75 ± 0.30 mm, p<0.0001; 2.20 ± 0.30 mm, p<0.0001), from baseline to 12 months at the treatment site, with no significant differences between groups (PD: p=0.716; CAL: p=0.260).

As summarized in Table 3, sites adjacent to the treatment sites saw smaller, but significant improvements in PD and CAL, with no significant differences between the groups (PD: p=0.799; CAL: p=0.743). The adjacent sites showed a PD reduction of 0.89 ± 0.16 mm, p<.0001 and gain in CAL of 0.66 ± 0.18 mm, p=0.001 in the PR/RP+EMD group and a PD reduction of 0.84 ± 0.16 mm, p<0.0001 and CAL gain of 0.59 ± 0.18 mm, p=0.002 in the PR/RP+S group over 12 months.

In Table 4, the direct buccal and lingual sites of the experimental teeth saw small, but statistically significant improvements for both groups in PD (PR/RP+EMD -0.44 mm ± 0.08 mm, p<0.0001; PR/RP+S -0.30 mm ± 0.08 mm, p=0.001) and a significant improvement in CAL in the saline control group, but not the EMD group. (PR/RP+EMD 0.24 mm ± 0.13 mm, p=0.082;

PR/RP+S 0.50 mm ± 0.13 mm, p<0.001); however, the differences between the groups were not significant (PD: p=0.198; CAL: p=0.144).

Interproximal sites opposite that of the treatment site (Table 5) also had a small, but significant improvement in PD (PR/RP+EMD -0.61 mm ± 0.11 mm, p<0.0001; PR/RP+S -0.48 mm ± 0.11 mm, p<0.0001) and CAL (PR/RP+EMD -0.59 mm ± 0.14 mm, p<0.0001; PR/RP+S -0.57 mm ± 0.13 mm, p<0.001) for both groups, with no significant differences between groups (PD: p=0.346; CAL: p=0.905). Opposite sites were defined as the opposite interproximal surface on the treatment tooth, i.e., if the treatment site was the mesial surface, the distal surface of that same tooth would be considered the opposite site.

No significant differences were found in IBH between groups at baseline for the treatment site (p=1.0) or the adjacent site (p=0.53). Both groups had stable IBH (Table 6) at the treatment site over 12 months (PR/RP+EMD -0.20 ± 0.18 mm, p=0.28; PR/RP+S -0.33 ± 0.18 mm, p=0.08), with no significant differences between the groups (p=0.61). Adjacent site bone heights also remained stable over the 12 month period in both groups (PR/RP+EMD -0.04 ± 0.16 mm, p=0.81; PR/RP+S -0.18 ± 0.18 mm, p=0.26), with no significant difference found between the groups (p=0.51).

Plaque index (Table 7) was calculated using 12 sites from the experimental teeth. Both PR/RP+EMD and PR/RP+S groups saw a reduction in the experimental teeth plaque index (PR/RP+EMD -23% ± 5%, p=0.0001; PR/RP+S -12% ± 5%, p=0.028), with no significant differences between the two groups (p=0.129). At the treatment site, the proportion of Emdogain patients with plaque at twelve months (58.3%) was significantly lower (p = 0.02), than the proportion at

baseline (87.5%). For saline patients, the proportion of patients with plaque at twelve months (66.7%), did not significantly differ ($p = 0.10$), from the proportion at baseline (87.5%), as referenced in Table 8.

TABLE 1: Differences in Demographics between Groups (Data used for randomization)
Statistic: Chi-Square

		Emdogain		Control		
		n	**%**	**n**	**%**	**P-Value**
Gender						0.40
	Female	13	54.2	11	42.3	
	Male	11	45.8	15	57.7	
Smoking Status						0.33
	Non-smoker	21	87.5	20	76.9	
	Smoker	3	12.5	6	23.1	
Mean Age		66.92 (±1.15)		64.96 (±2.06)		0.41

*Indicates change was significant ($p < 0.05$)

Interpretation: The distribution of men and women, or smoker and non-smokers, and age was not significantly different between groups.

Table 2: Clinical Outcomes at Treatment Site
Statistics: T-test, Wilcoxon Rank Sums test, Signed Rank test

Variable	EMD Baseline Mean (mm ± SE)	Saline Control Baseline Mean (mm ± SE)	T-test p-value	Model Adjusted EMD Mean Change (Final - Initial) (mm ± SE) **	P-value for EMD Mean Change (Change Over Time)	Model Adjusted Saline Control Mean Change (Final - Initial) (mm ± SE) **	P-value for Saline Control Mean Change (Change Over Time)	P-value for Variable (Differences Between Groups)
PD	6.88 (0.24)	6.46 (0.15)	0.14	-2.29 (0.21)	<0.0001*	-2.39 (0.21)	<0.0001*	0.716
CAL	7.58 (0.28)	7.35 (0.3)	0.58	-1.75 (0.30)	<0.0001*	-2.20 (0.30)	<0.0001*	0.260

*Indicates change was significant ($p < 0.05$)
**Negative number indicates postoperative improvement

Interpretation: After controlling for the initial measurement and side with deepest pocket, significant changes were noted in both the EMD and saline groups for PD and CAL at the treatment site. There were no significant differences in the changes observed between the two groups.

Table 3: Clinical Outcomes at Adjacent Site
Statistics: T-test, Wilcoxon Rank Sums test, Signed Rank test

Variable	EMD Baseline Mean (mm ± SE)	Saline Control Baseline Mean (mm ± SE)	T-test p-value	Model Adjusted EMD Mean Change (Final - Initial) (mm ± SE) **	P-value for EMD Mean Change (Change Over Time)	Model Adjusted Saline Control Mean Change (Final - Initial) (mm ± SE) **	P-value for Saline Control Mean Change (Change Over Time)	P-value for Variable (Differences Between Variable Groups)
PD	4.23 (0.24)	4.44 (0.22)	0.53	-0.89 (0.16)	<0.0001*	-0.84 (0.16)	<0.0001*	0.799
CAL	4.83 (0.33)	5.06 (0.25)	0.58	-0.66 (0.18)	0.001*	-0.59 (0.18)	0.002*	0.743

*Indicates change was significant ($p < 0.05$)
**Negative number indicates postoperative improvement

Interpretation: After controlling for the initial measurement and side with deepest pocket, significant changes were noted in both the EMD and saline groups for PD and CAL at the interproximal site adjacent to the treatment site. There were no significant differences in the changes observed between the two groups.

Table 4: Clinical Outcomes at Direct Buccal and Lingual Sites
Statistics: T-test, Wilcoxon Rank Sums test, Signed Rank test

Variable	EMD Baseline Mean (mm ± SE)	Saline Control Baseline Mean (mm ± SE)	T-test p-value	Model Adjusted EMD Mean Change (Final - Initial) (mm ± SE) **	P-value for EMD Mean Change (Change Over Time)	Model Adjusted Saline Control Mean Change (Final - Initial) (mm ± SE) **	P-value for Saline Control Mean Change (Change Over Time)	P-value for Variable (Differences Between Variable Groups)
PD	2.65 (0.12)	2.61 (0.1)	0.84	-0.44 (0.08)	<0.0001*	-0.30 (0.08)	0.001*	0.198
CAL	3.73 (0.25)	3.89 (0.23)	0.65	-0.24 (0.13)	0.082	-0.50 (0.13)	0.001*	0.144

*Indicates change was significant ($p < 0.05$)
**Negative number indicates postoperative improvement

Interpretation: After controlling for the initial measurement and side with deepest pocket, significant reductions were noted in both the EMD and saline groups for PD on the direct buccal and lingual of the treatment tooth. A significant change in CAL was found in the saline group, but not the EMD group. There were no significant differences in the changes observed between the two groups.

Table 5: Clinical Outcomes at Opposite Sites
Statistics: T-test, Wilcoxon Rank Sums test, Signed Rank test

Variable	EMD Baseline Mean (mm ± SE)	Saline Control Baseline Mean (mm ± SE)	T-test p-value	Model Adjusted EMD Mean Change (Final - Initial) (mm ± SE) **	P-value for EMD Mean Change (Change Over Time)	Model Adjusted Saline Control Mean Change (Final - Initial) (mm ± SE) **	P-value for Saline Control Mean Change (Change Over Time)	P-value for Variable (Differences Between Variable Groups)
PD	3.77 (0.18)	3.98 (0.2)	0.45	-0.61 (0.11)	<0.0001*	-0.48 (0.11)	<0.0001*	0.346
CAL	4.47 (0.24)	4.53 (0.25)	0.86	-0.59 (0.14)	<0.0001*	-0.57 (0.13)	<0.0001*	0.905

*Indicates change was significant ($p < 0.05$)
**Negative number indicates postoperative improvement

Interpretation: After controlling for the initial measurement and side with deepest pocket, significant changes were noted in both the EMD and saline groups for PD and CAL at the site opposite of the treatment site on the treatment tooth. There were no significant differences in the changes observed between the two groups.

Table 6: Interproximal Bone Height Outcomes
Statistics: T-test, Wilcoxon Rank Sums test, Signed Rank test

Variable	EMD Baseline Mean (mm ± SE)	Saline Control Baseline Mean (mm ± SE)	T-test p-value	Model Adjusted EMD Mean Change (Final - Initial) (mm ± SE) **	P-value for EMD Mean Change (Change Over Time)	Model Adjusted Saline Control Mean Change (Final - Initial) (mm ± SE) **	P-value for Saline Control Mean Change (Change Over Time)	P-value for Variable (Differences Between Variable Groups)
Treat-ment Site	5.1 (0.36)	5.09 (0.37)	1.0	-0.20 (0.18)	0.28	-0.33 (0.18)	0.08	0.61
Adjacent Site	4.51 (0.43)	4.87 (0.38)	0.53	-0.04 (0.16)	0.81	-0.18 (0.18)	0.26	0.53

*Indicates change was significant ($p < 0.05$)
**Negative number indicates postoperative improvement

Interpretation: No significant changes were seen in IBH in the EMD or saline groups at either the treatment site or the adjacent site, from baseline to 12 months. No significant differences were found between groups.

Table 7: Plaque Index of Experimental Teeth

Variable	EMD Baseline Mean (± SE)	Saline Control Baseline Mean (± SE)	T-test p-value	Model Adjusted EMD Mean Change (Final - Initial) (± SE) **	P-value for EMD Mean Change (Change Over Time)	Model Adjusted Saline Control Mean Change (Final - Initial) (± SE) **	P-value for Saline Control Mean Change (Change Over Time)	P-value for Variable (Differences Between Variable Groups)
Plaque	66% (7%)	67% (5%)	0.90	-23% (5%)	0.0001*	-12% (5%)	0.028*	0.129

*indicates change was significant ($p < 0.05$)
**Negative number indicates postoperative improvement

Interpretation: Data for experimental teeth plaque index was computed using the mean of six sites from the treatment tooth and six sites from the adjacent tooth, for a total of 12 sites. Significant reductions were seen in the plaque indices in both the EMD and saline control groups from baseline to 12 months, with no significant differences in the changes observed between groups ($p = 0.129$).

Table 8: Change in Percent of Plaque at Treatment Site
Statistics: McNemar's Test and Chi-Square

	Baseline	**Post-Treatment**	**p-Value**
EMD	87.5%	58.5%	0.02*
Saline Control	87.5%	66.7%	0.10

*indicates change was significant ($p < 0.05$)

Interpretation: For EMD patients, the proportion of patients with plaque at twelve months was significantly lower than the proportion at baseline ($p = 0.02$). For saline patients, the proportion of patients with plaque at twelve months did not significantly differ from the proportion at baseline ($p = 0.10$)

CHAPTER 7: DISCUSSION

This double-blinded, randomized, controlled clinical trial compared clinical outcomes of two therapies, PR/RP+EMD (test) and PR/RP+S (control), in 6-9 mm pockets with a history of BOP in periodontal maintenance patients over a 12 month period. Every precaution was taken to eliminate any bias by compartmentalizing the various aspects of this study protocol as follows: masked examiners (AK, RR, JP) collected all data, patients were masked to treatment group assignment, treatment was performed by one of two clinicians (EJ, JG) with the assistance of the same dental hygienist (MC), one dental hygienist performed all maintenance procedures (MC), and randomization was done after PR/RP was completed to minimize any bias in thoroughness of debridement. All of the participants in this study were on a three month periodontal maintenance recall program and had previously received regular PMT. There were no significant differences in the baseline clinical measurements between the PR/RP+EMD and PR/RP+S sites. The manufacturer of Emdogain did not support the study in any way.

The primary outcome measure in this study was a change in CAL, with change in IBH, PD, and PL as secondary outcome measures. The current study demonstrated that inflamed, 6-9 mm pockets, treated with PR/RP+EMD or PR/RP+S showed gain in CAL, PD reduction, and stability of IBH 12 months post-therapy in a periodontal maintenance population. No significant differences were found between the groups when comparing clinical measurements of PR/RP+EMD to PR/RP+S at baseline or 12 months. To our knowledge, no other studies have compared clinical measures of PR/RP+EMD to PR/RP+S during periodontal maintenance procedures.

The current study showed a significant gain in CAL in both the experimental and control groups. The PR/RP+EMD group showed a gain of 1.75 ± 0.3 mm and the PR/RP+S group showed a gain of 2.2 ± 0.3 mm over 12 months. The difference between the groups was not statistically significant (p=0.260). The improvements seen in CAL in this study are slightly better than those reported in previous studies during initial therapy, with reported gains of 1.29 mm in PD >7 mm (Cobb, 1996).

Patients who are not enrolled in regular PMT experience a significant amount of alveolar bone loss compared to those who receive regular PMT (Becker et al., 1984). Previous studies have indicated patients who receive regular PMT, average less than 1mm of alveolar bone height changes over a 14 year period (Lindhe and Nyman, 1984). These numbers are in line with changes in alveolar bone height found in this study. The mean changes of approximately 0.2 to 0.3 mm of alveolar bone height changes seen in the current study are short of the 1mm change in bone height needed for reproducibility when evaluating a series of radiographs (Hausmann et al., 1997). Results from the present study indicate stable alveolar bone height in both groups at 12 months, with no significant differences between the groups (p=0.61). This is presumably due to the study population having been enrolled in a PMT program.

The improvements seen in PD over the course of the study were similar to the 2.16 mm PD reduction reported by Cobb (1996), in pocket depths >7 mm following SRP. In the current study, a PD reduction of 2.29 ± 0.21 mm, $p<0.0001$ was seen in the PR/RP+EMD group and a reduction of 2.39 ± 0.21 mm, $p<.0001$ in the PR/RP+S group. These results indicated that the same benefit may be achieved from SRP and PR/RP alone. Therefore, the addition of EMD did

not provide an enhanced PD reduction. These results are in line with those found by Gutierrez et, al., (2003) comparing the addition of EMD to SRP with SRP alone. That study reported a PD reduction of 2.3 ± 0.5 mm in the control (SRP) sites and 2.0 ± 0.3 mm in the experimental (SRP+EMD) sites, with no significant differences between the groups ($p>0.4$). The conclusion from that study, similar to the conclusion of the current study, did not support the use of EMD during routine root planing.

Plaque control was poor in the recruited maintenance patients for the current study. Plaque was present at a majority of treatment sites at both baseline (87%) and 12 month (PR/RP+EMD 58.3%; PR/RP+S 66.7%) post-therapy exam. Although the improvements in PL were significant for both groups, only a small improvement in PL was seen over the course of the study. The high supragingival plaque levels reported in the current study were similar to those found by Reinhardt et al. (2007), who showed 56%-68% explorer-detectable plaque levels across time points throughout a study evaluating posterior interproximal sites and the use of systemic sub-antimicrobial dose doxycycline. All of the studied treatment sites were posterior, interproximal sites which have been shown to be difficult for patients to clean effectively (Cumming & Loe, 1973; Sherman et al., 1990; Prasad et al., 2011). Improved PL levels may have resulted in better clinical results in the current study for both groups. OHI was reviewed at every appointment; however, the EMD protocol requiring no brushing or interproximal cleaning of the treatment site for six weeks may have given the patients the impression they could do harm by cleaning the site, even once it was allowed. Results from previous studies have indicated the stability of results gained following regenerative periodontal therapy are dependent on compliance with PMT and stringent oral hygiene (Cortellini et al., 1996; Rasperini

et al., 2005). A plaque index <35% is recommended for periodontal stability (Purschwitz et al., 2008), as renewed accumulation of plaque in the operated areas may result in recurrence of periodontal disease including a significant further loss of attachment (Nyman et al., 1977). In addition, any explorer-detectable plaque was registered as positive in the current index, potentially overestimating of plaque levels compared to other studies.

Many of the current studies evaluating the use of EMD were done utilizing surgical access. Most of these studies showed a significantly greater improvement in clinical parameters with the use of EMD (Heijl et al., 1997; Pontoriero et al., 1999; Froum et al., 2001). The study by Heijl et al. (1997), compared the long-term effects of EMD as an adjunct to MWF and demonstrated PD reduction of 3.1 mm in the EMD group and 2.3 mm in the placebo group at 36 months ($p<0.001$); and gains in CAL of 2.2 mm with EMD and gains of 1.7 mm for placebo at 36 months ($p<0.01$). Pontoriero et al. (1999), reported a 4.4 mm reduction in PDs in sites treated with open flap debridement (OFD) + EMD compared to a 3.5 mm reduction in OFD alone ($p<0.001$); and a 3 mm improvement in attachment level with the addition of EMD, compared to 1.8 mm gain with OFD alone ($p<0.001$). Froum (2001), also compared OFD + EMD to OFD alone and showed a statistically significant difference in gain of attachment (1.5 mm) in favor of the addition of EMD. These improvements are better than those found in the current study. Surgical access allows for a more thorough debridement of the root surface and less residual calculus than a closed, non-surgical approach (Caffesse et al., 1986). Surgical access also allows for an environment with reduced blood, moisture, and debris, which may ensure the root surface is adequately coated with the EMD (Gutierrez et al., 2003). When EMD is applied in a non-surgical environment, adequate coating of the root surface and containment of the EMD

cannot be guaranteed (Gutierrez et al., 2003). In fact, some studies have shown approximately 50% of a medicament (chlorhexidine and stannous fluoride gels) applied subgingivally may be flushed out of a pocket immediately after insertion (Oosterwaal et al., 1990). This high clearance rate may affect the amount of EMD which was able to remain in contact with the root surface in order to see a benefit. Maintenance of a dry, bloodless field during EMD application is recommended by the manufacturer to achieve the best results. These conditions were hard to achieve in the current study as the use of a localized papilla reflection did not allow for complete isolation of the experimental site. This may explain the differences in the clinical outcomes reported by the current study, compared to those reported in studies with more extensive surgical techniques than described above. Alternatively, the use of the endoscope may have improved the root debridement sufficiently to where EMD could not provide further impact.

In contrast to the current study, some studies utilizing a similar minimally invasive surgical technique with papilla reflection and EMD application showed significant reductions in clinical parameters with the use of EMD (Harrel et al, 2005; Cortellini & Tonetti, 2007; Cortellini et al., 2008). However, these results must be interpreted with caution as these studies were performed without a control. In a study by Harrel et al. (2005), surgical treatment was performed using a minimally invasive technique and the addition of EMD in 160 sites. Mean PD reductions ($p=0.002$) and CAL improvements ($p=0.012$) were significantly greater than 3 mm, with mean post-surgical PDs of 3.17 mm and attachment levels of 4.05 mm. A study by Cortellini & Tonetti (2007), utilized a minimally invasive, papilla preservation, surgical technique with an operating microscope, microsurgery instruments, and the addition of EMD to debrided

surfaces in 13 infrabony pockets. The one year CAL gain was 4.8 ± 1.9 mm. Residual PDs were 2.9 ± 0.8 mm. Differences between baseline and one year CAL and PD were both clinically and statistically significant ($p<0.0001$). The one year percent resolution of the defect was 88.7 ± 20.7%, and reached 100% of the baseline intra-bony component in seven sites. In a follow-up study, a similar protocol was used on a larger sample size (44) and showed similar improvements. The one year CAL gain was 4.4 ±1.4 mm ($p<0.0001$ compared with baseline). Seventy-three per cent of defects showed CAL improvements ≥4 mm. This corresponded to an 83 ± 20% resolution of the defect (15 defects were completely filled). Residual PDs were 2.5 ± 0.6 mm (Cortellini et al., 2008). The major difference between the current study and the Cortellini & Tonetti (2007) and Cortellini et al., (2008), studies were the site characteristics. The current study evaluated the effect of EMD with a papilla reflection technique in sites with primarily horizontal bone loss, as confirmed by only a 0.4 mm mean difference in IBH between treatment and adjacent sites (Table 6), with intervention being performed during a periodontal maintenance appointment, whereas the two previously mentioned studies evaluated infrabony defects during dedicated and more extensive surgery appointments. This may play a factor in the amount of regeneration which occurred, as defects with a depth of >3 mm and a radiographic defect angle of 25 degrees were reported to be the most amenable to regenerative procedures (Tonetti et al., 2003). Non-supportive anatomy, defined as predominantly a one wall defect, is a risk factor for failure of regeneration attempts (Cosyn et al., 2012). The study by Harrel (2005), was performed at multiple practices and the mean results were presented. The data from this study may have been skewed, as one practice combined EMD with freeze-dried demineralized bone, thereby adding another factor and preventing direct comparisons. The

Harrel study was also funded by Straumann, the manufacturer of EMD. The current study added 30 minutes of the PMT appointment, which patients tended to accept more readily than a separate "surgery" visit. Outcomes resulted in mean probing depths around 4mm with access for interproximal brushes, and very low patient morbidity (most patients reported no use of analgesics postoperatively).

Improvements in clinical outcomes (PD and CAL) were seen in the current study at sites other than the treatment site (adjacent, direct buccal and lingual, and opposite). Most of these improvements were small (<1 mm). While these were statistically significant, the clinical ramifications are minimal and well within measurement error of $\pm$ 1 mm (Osborn et al, 1992; Corrainin et al., 2013). These improvements may also be contributed to the Hawthorne Effect, and the patients' improvement in homecare due to knowledge of participating in a clinical study.

This study has several limitations. Patients were observed for a period of only 12 months and a longer duration would be ideal to determine long term stability. Also, the history of each study site was not investigated, some may have been experiencing active attachment loss while others may have been periodontally stable. This may have influenced the response to the treatment and the inflammatory condition of the pockets. Although a control was used in this study, both treatment modalities incorporated the use of the endoscope. The enhanced visualization provided by the endoscope may have reduced the effect which would have been seen between the two treatments, due to the superior root debridement that it allowed.

However, with clinical improvements seen in both groups, this may indicate thorough root debridement is a more important factor than the addition of EMD.

Many adjunctive therapies (local chemotherapeutics, lasers, biologics, etc.) are being used to try to enhance the clinical benefits encountered with both non-surgical and surgical periodontal therapies. More research is needed to determine the clinical benefits, as well as the cost benefit of these adjunctive therapies. The American Academy of Periodontology (AAP) stated that the use of adjuncts is not proven to "reduce the need for surgery or improve long-term tooth retention," or to be cost effective (AAP Statement 2006). The AAP addressed the limitations of adjunctive therapies and stated that local adjuncts resulted in modest improvements (PD reduction of 0.25-0.5 mm) in the clinical outcomes of pockets ≥ 5 mm. Much of the research surrounding EMD as an adjunct to periodontal debridement/surgery is conflicting, has been sponsored by the manufacturer, or lacks controls. Additional research is needed to determine the clinical benefits of EMD, its regenerative ability, and how to best implement the use of EMD to enhance the treatment of periodontitis. Further research is also needed to determine EMD's effect on inflammatory biomarkers, the reduction of the subgingival bacterial flora, and how these correlate with an improvement in the clinical manifestations of periodontitis.

CHAPTER 8: CONCLUSION

Scaling and root planing with papilla reflection in inflamed, persistent, deep periodontal pockets during PMT with or without the addition of Emdogain, resulted in improvements in PD, and CAL, with stable IBH after 12 months of PMT. The addition of EMD does not enhance the clinical benefits of PR/RP in the treatment of periodontal maintenance patients with inflamed 6-9 mm probing depths.

Due to the amount of conflicting data regarding the use of EMD, further studies comparing PR/RP+EMD to other adjunctive therapies should be pursued. Additionally, future research could be done to assess the anti-microbial and anti-inflammatory effects of EMD in periodontal maintenance patients.

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Appendix A: Patient Consent Form

University of Nebraska Medical Center

College of Dentistry

IRB PROTOCOL # 783-16-FB **Page 1 of 8**

ADULT CONSENT - CLINICAL BIOMEDICAL

Title of this Research Study

Effect of Enamel Matrix Proteins on Clinical Attachment Level and Alveolar Bone in Periodontal Maintenance Patients

Invitation

You are invited to take part in this research study. You have a copy of the following, which is meant to help you decide whether or not to take part:

- Informed consent form
- "What Do I need to Know Before Being in a Research Study?"
- The Rights of Research Subjects

Why are you being asked to be in this research study?

You are being asked to be in this study because you are 40-85 years old and have periodontal disease. If you are pregnant, nursing an infant, or plan to become pregnant during this study, you may not be in this study.

What is the reason for doing this research study?

The scientific purpose of this study is to determine the effect of enamel matrix proteins in patients with periodontal bone loss who are on a regular periodontal maintenance routine.

Enamel matrix protein derivative is an FDA-approved drug for the purpose of bone regeneration in periodontal bone defects. This study is using enamel matrix protein derivative for an on-label purpose.

What will be done during this research study?

If selected for this study, you will be randomized (similar to flipping a coin) to one of the two study groups. One study group will receive a dose of enamel-matrix protein derivative (FDA-approved) to a site with a deep periodontal pocket. The other study group will receive a dose of sterile saline to a site with a deep periodontal pocket.

You will have a radiograph (x-ray) taken of the tooth with the deep pocket. Probe depths will be taken of the study site. Small paper points will be used to soak up the fluid from within the deep pocket. You will then receive a small injection of local anesthesia, followed by lifting of the gum tissue in the area of the deep pocket. The study drug (enamel matrix protein derivative) or sterile saline will be placed in the deep pocket after it has been cleaned. The gum tissue will be put back into place and secured with dental glue. The rest of the teeth will then be cleaned as typical of a periodontal maintenance appointment. You will be seen approximately two weeks

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later to make sure that the study site is healing well. You will continue on your typical periodontal maintenance schedule and will have a new radiograph and new sample of fluid taken of the study tooth in 12 months.

The initial study visit will include review of the informed consent, taking an x-ray, measurements and sampling of fluid from study site, small injection of anesthesia, lifting of gum tissue, placement of the study drug or saline, closure of the study site with dental glue and cleaning of all remaining teeth in the mouth (periodontal maintenance therapy) and will take approximately 90-120 minutes.

At the two-week follow-up visit, the study site will be checked for proper healing and to answer any questions. This visit will take approximately 15-30 minutes.

The 3, 6, and 9-month periodontal maintenance visits will be simple, routine maintenance appointments that will include full-mouth probing of teeth, cleaning of all teeth, polishing and flossing. These appointments take approximately 45 minutes.

The 12-month appointment will include a radiograph and fluid sample of the study tooth, full-mouth probing of all teeth followed by routine periodontal maintenance (cleaning, polishing, flossing) of all teeth. This appointment will take approximately 60 minutes.

If you are a female, prior to all visits, you will be asked to complete a urine pregnancy test.

Information recorded at each visit will include periodontal probing depth, recession and bleeding on probing.

What are the possible risks of being in this research study?
No risks are associated with the paper strip. The enamel matrix protein derivative is FDA-approved for treatment of periodontal pockets via a surgical approach used over past 20 years. The mini-flap and EMD application are within the standard of care for 6-9 mm inflamed periodontal pockets (inclusion criteria). The potential risks associated with the enamel matrix protein derivative are no different than those associated with routine surgical periodontal therapy: 1) local infection, 2) mild-moderate discomfort, 3) bleeding.

The risk of loss of confidentiality is low due to the few number of investigators as well as the privacy of the office where consent will be obtained and the clinic where the data will be collected. The locked filing cabinet and locked room in the Cruzan Center

where the data will be kept will also contribute to a low risk of loss of confidentiality.

It is possible that the medicines used in this study could injure a fetus if you, or your partner, becomes pregnant while taking them. You have already been told what is known about this possibility, and you are encouraged to ask further questions.

You may want to discuss this with others before you agree to take part in this study. If you wish, we will arrange for a doctor, nurse, or counselor who is not part of this study to discuss the potential risks and benefits with you and anyone else you want to have present.

Because of the potential risks, you, or your partner, must not become pregnant while you are participating in this study. Women must have a negative pregnancy test before entering the study and before each treatment.

If you are sexually active and can get pregnant, or can get your partner pregnant, you must use TWO appropriate methods of birth control every time you have sex, or you must not have sex.

Because of the nature of this research, methods of natural family planning are not, by themselves, sufficiently reliable to avoid pregnancy.

You can get additional information about methods to avoid pregnancy by calling the UNMC Research Subject Advocate's Office at (402) 559-6941.

By signing this and being in the study, you are agreeing to not get pregnant while you are on the study and for 6 months after completion of the study.

It is possible that other rare side effects could occur which are not described in this consent form. It is also possible that you could have a side effect that has not occurred before. Should you become pregnant while on this study, you should immediately notify the study personnel. The investigator will assist you in finding appropriate medical care. The investigator also may ask to be allowed to continue getting information about your pregnancy. You can refuse to provide this information.

What are the possible benefits to you?
Possible benefits of being in this study could include bone regrowth or a decreased pocket in the area of the deep pocket.

What are the possible benefits to other people?

This study could possibly benefit society by examining another way to treat deep pockets and periodontal bone loss, which could ultimately lead to patients retaining their own teeth for a longer time.

What are the alternatives to being in this research study?
The alternative to being in this study would be periodontal surgery with or without chemotherapeutics. In addition, subjects could receive EMD in a non-research setting.

What will being in this research study cost you?
There is no cost to you to be in this research study.

Will you be paid for being in this research study?
You will not be paid to be in this research study. You will receive your periodontal maintenance therapy for half fee while in this study (12 months).

Who is paying for this research?
This research is being paid for by the Department of Surgical Specialties, Section of Periodontics at the University of Nebraska Medical Center College of Dentistry.

This research is being paid for by grant funds from the Windsweep Farm Fund.

What should you do if you are injured or have a medical problem during this research study?
Your welfare is the main concern of every member of the research team. If you are injured or have a medical problem as a direct result of being in this study, you should immediately contact one of the people listed at the end of this consent form. Emergency medical treatment for this injury or problem will be available at the Nebraska Medical Center. If there is not sufficient time, you should seek care from a local health care provider.

The Institution has no plans to pay for any required treatment or provide other compensation. If you have insurance, your insurance company may or may not pay the costs of medical treatment. If you do not have insurance, or if your insurance company refuses to pay, you will be expected to pay for the medical treatment.

Agreeing to this does not mean you have given up any of your legal rights.

How will information about you be protected?
You have rights regarding the protection and privacy of your medical information

collected before and during this research. This medical information is called "protected health information" (PHI). PHI used in this study may include your medical record number, address, birth date, medical history, the results of physical exams, blood tests, x-rays as well as the results of other diagnostic medical or research procedures. Only the minimum amount of PHI will be collected for this research. Your research and medical records will be maintained in a secure manner.

Who will have access to information about you?
By signing this consent form, you are allowing the research team to have access to your PHI. The research team includes the investigators listed on this consent form and other personnel involved in this specific study at the Institution.

Your PHI will be used only for the purpose(s) described in the section What is the reason for doing this research study?

You are also allowing the research team to share your PHI, as necessary, with other people or groups listed below:

- The UNMC Institutional Review Board (IRB)
- Institutional officials designated by the UNMC IRB
- Federal law requires that your information may be shared with these groups:
    - The HHS Office of Human Research Protections (OHRP)
    - The Food and Drug Administration

You are authorizing us to use and disclose your PHI for as long as the research study is being conducted.

You may cancel your authorization for further collection of PHI for use in this research at any time by contacting the principal investigator in writing. However, the PHI which is included in the research data obtained to date may still be used. If you cancel this authorization, you will no longer be able to participate in this research.

How will results of the research be made available to you during and after the study is finished?
Information obtained in the course of the research that will not be shared with you is in to which group (study drug versus saline) you have been randomized. By signing this authorization, you are temporarily giving up your right to see this research-related information while the research is going on. You will be able to see this information if you wish after the research is completed.

In most cases, the results of the research can be made available to you when the

study is completed, and all the results are analyzed by the investigator or the sponsor of the research. The information from this study may be published in scientific journals or presented at scientific meetings, but your identity will be kept strictly confidential.

If you want the results of the study, contact the Principal Investigator at the phone number given at the end of this form or by writing to the Principal Investigator at the following address: Dr. Amy C. Killeen, UNMC College of Dentistry, 40th and Holdrege, Lincoln NE

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by U.S. law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

What will happen if you decide not to be in this research study?
You can decide not to be in this research study. Deciding not to be in this research will not affect your dental care or your relationship with your dental providers or the College of Dentistry. Your dentist or hygienist will still take care of you and you will not lose any benefits to which you are entitled.

What will happen if you decide to stop participating once you start?
You can stop participating in this research (withdraw) at any time by contacting the Principal Investigator or any of the research staff. Deciding to withdraw will otherwise not affect your care or your relationship with the investigator or this institution.

You could be withdrawn from the study if you show any signs of allergy or hypersensitivity, become pregnant, or demonstrate rapidly progressing periodontitis in the experimental quadrant requiring periodontal surgery or tooth extractions or if you are non-compliant with the required visits. Any research data obtained to date may still be used in the research.

Will you be given any important information during the study?
You will be informed promptly if the research team gets any new information during this research study that may affect whether you would want to continue being in the study.

What should you do if you have any questions about the study?
You have been given a copy of "*What Do I Need to Know Before Being in a Research Study?*" If you have any questions at any time about this study, you should

contact the Principal Investigator or any of the study personnel listed on this consent form or any other documents that you have been given.

What are your rights as a research participant?
You have rights as a research subject. These rights have been explained in this consent form and in The Rights of Research Subjects that you have been given. If you have any questions concerning your rights, or want to discuss problems, concerns, obtain information or offer input, or make a complaint about the research, you can contact any of the following:

- The investigator or other study personnel
- Institutional Review Board (IRB)
    - Telephone: (402) 559-6463.
    - Email: IRBORA@unmc.edu
    - Mail: UNMC Institutional Review Board, 987830 Nebraska Medical Center, Omaha, NE 68198-7830
- Research Subject Advocate
    - Telephone: (402) 559-6941
    - Email: unmcrsa@unmc.edu

Documentation of informed consent
You are freely making a decision whether to be in this research study. Signing this form means that:

- You have read and understood this consent form.
- You have had the consent form explained to you.
- You have been given a copy of The Rights of Research Subjects
- You have had your questions answered.
- You have decided to be in the research study.
- If you have any questions during the study, you have been directed to talk to one of the investigators listed below on this consent form.
- You will be given a signed and dated copy of this consent form to keep.

Signature of Subject _____
Date _____

My signature certifies that all the elements of informed consent described on this consent form have been explained fully to the subject. In my judgment, the subject possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate.

Signature of Person obtaining consent _____
Date _____

Authorized Study Personnel
Principal
* Killeen, Amy
phone: 402-472-1441
alt #: 402-202-1174
degree: DDS

Secondary
* Payne, Jeffrey
phone: 402-472-1318
alt #: 402-472-1318
degree: DDS, M. Dent.Sc

* Reinhardt, Richard (Rick)
phone: 402-472-1287
alt #: 402-472-1287
degree: DDS

Institutional Review Board (IRB)

What Do I Need To Know Before Being In A Research Study?

You have been invited to be in a **research study**. Research studies are also called "clinical trials" or "protocols." **Research** is an organized plan designed to get new knowledge about a disease or the normal function of the body. The people who are in the research are called **research subjects**. The **investigator** is the person who is running the research study. You will get information from the investigator and the research team, and then you will be asked to give your **consent** to be in the research.

This sheet will help you think of questions to ask the investigator or his/her staff. You should know all these answers before you decide about being in the research.

What is the **purpose** of the research? Why is the investigator doing the research?

What are the **risks** of the research? What bad things could happen?

What are the possible **benefits** of the research? How might this help me?

How is this research different than the care or treatment I would get if I wasn t in the research? Are there other treatments I could get?

Does **everyone** in this research study get the same treatment?

Will being in the research **cost** me anything extra?

Do I have to be in this research study? Will the doctor still take care of me if I say **no**?

Can I **stop** being in the research once I ve started? How?

Who will look at my **records**?

How do I reach the investigator if I have more **questions**?

Who do I call if I have questions about being a **research subject**?

Make sure all your questions are answered before you decide whether or not to be in this research.

Academic Research & Services Building 3000 / 987830 Nebraska Medical Center / Omaha NE 68198-7830
402-559-6463 / FAX 402-559-3300 / Email: irbora@unmc.edu / http://www.unmc.edu/irb

Institutional Review Board (IRB)

THE RIGHTS OF RESEARCH SUBJECTS
AS A RESEARCH SUBJECT YOU HAVE THE RIGHT

to be told everything you need to know about the research before you are asked to decide whether or not to take part in the research study. The research will be explained to you in a way that assures you understand enough to decide whether or not to take part.

to freely decide whether or not to take part in the research.

to decide not to be in the research, or to stop participating in the research at any time. This will not affect your medical care or your relationship with the investigator or the Nebraska Medical Center. Your doctor will still take care of you.

to ask questions about the research at any time. The investigator will answer your questions honestly and completely.

to know that your safety and welfare will always come first. The investigator will display the highest possible degree of skill and care throughout this research. Any risks or discomforts will be minimized as much as possible.

to privacy and confidentiality. The investigator will treat information about you carefully, and will respect your privacy.

... to keep all the legal rights you have now. You are not giving up any of your legal rights by taking part in this research study.

to be treated with dignity and respect at all times

The Institutional Review Board is responsible for assuring that your rights and welfare are protected. If you have any questions about your rights, contact the Institutional Review Board at (402) 559-6463.

Academic Research & Services Building 3000 / 987830 Nebraska Medical Center / Omaha NE 68198-7830
402-559-6463 / FAX 402-559-3300 / Email: irbora@unmc.edu / http://www.unmc.edu/irb

Appendix B: Raw Clinical Data

Patient	Group	Age	Gender	Smoking	Baseline Treatment Site Buccal PD	Baseline Treatment Site Lingual PD	Baseline Adjacent Site Buccal PD
N1	2	59	1	1	7	6	4
N2	1	67	2	0	6	6	5
N3	1	74	1	0	9	5	3
N4	2	70	2	0	4	7	3
N5	1	67	2	0	7	5	3
N6	1	76	1	1	4	6	4
N7	2	55	1	0	6	7	4
N8	2	63	2	0	5	7	3
N9	2	80	2	0	5	6	4
N10	1	69	1	0	6	8	3
N11	1	62	1	0	7	8	4
N12	2	67	1	0	7	7	3
N13	2	76	2	0	6	5	4
N14	1	58	2	1	5	8	3
N15	2	83	2	0	4	6	8
N16	1	68	2	0	5	6	6
N17	2	47	1	0	6	5	5
N18	2	44	1	1	5	6	4
N19	2	65	1	1	4	6	3
N20	1	67	1	0	4	9	3
N21	1	59	2	0	8	7	5
N22	1	65	1	0	4	6	3
N23	1	80	2	0	3	6	3
N24	2	62	1	1	6	6	6
N25	1	65	2	0	4	6	3
N26	1	62	1	0	7	9	4
N27	1	67	2	1	6	4	3
N28	2	75	2	0	5	6	3
N29	2	76	1	0	6	5	5
N30	1	62	2	0	6	6	3
N31	2	68	1	0	6	7	6
N32	2	69	1	1	5	6	2
N33	2	66	1	1	6	7	5
N34	2	52	2	0	8	9	5

N35	2	60	1	0	5	6	4
N36	1	69	2	0	3	6	5
N37	2	79	1	0	5	7	5
N38	1	69	1	0	5	6	5
N39	2	65	2	0	6	6	3
N40	2	73	1	0	6	6	4
N41	2	67	2	0	6	6	4
N42	2	73	2	0	4	6	4
N43	1	68	2	0	6	7	6
N44	2	78	1	0	4	6	4
N45	2	48	2	0	5	6	4
N46	1	58	1	0	6	4	5
N47	1	61	2	0	6	6	6
N48	1	75	1	0	8	6	2
N49	1	68	1	0	4	6	4
N50	1	70	2	0	5	6	4
	1 - EMD		1 - male	0 - non-smoker			
	2 - saline		2 - female	1 - smoker			

Patient	Baseline Adjacent Site Lingual PD	Baseline Average Direct B&L Treatment Tooth PD	Average Direct B&L Adjacent Tooth PD	Average Treatment Tooth Opposite Site PD	Average Adjacent Tooth Opposite Site PD	Baseline Treatment Site Buccal REC
N1	4	2.5	3	4.5	6.5	1
N2	6	2.5	2.5	3.5	4	0
N3	3	2	2	4.5	2.5	0
N4	4	3	3	3.5	3.5	2
N5	3	3	2	4	3	1
N6	4	4	2.5	4	4	0
N7	4	3	2	3	4	0
N8	5	2.5	2	4	4	0
N9	6	2.5	2.5	2.5	4	0
N10	6	3.5	3.5	5	7	0
N11	4	2.5	2.5	4	3.5	0
N12	3	2.5	2	2.5	3	3
N13	4	2	2.5	2	3	0
N14	3	2	2	5	3.5	0
N15	4	2.5	2.5	3	3	0
N16	6	2	4	3	3	4
N17	6	3	2.5	6	4.5	2
N18	6	2	2.5	3	2.5	1
N19	3	3	2	5.5	3	0
N20	5	5	2	3.5	3	1
N21	5	2	2	2	2.5	2
N22	3	2	2.5	3	3	0
N23	3	2.5	2	3	3	0
N24	8	2.5	2.5	5.5	5.5	0
N25	4	3.5	2	4	3	0
N26	6	2.5	3	6	3.5	0
N27	2	2	2	2.5	3	2
N28	5	2	2	3.5	3.5	1
N29	3	2.5	3	3	3	1
N30	3	2	2.5	5	4.5	2
N31	7	4.5	3	4	3.5	0
N32	3	4	2	2	4	3
N33	6	3.5	3.5	4	3.5	0
N34	6	3	3	3	7	0
N35	4	2	2	3.5	4.5	0
N36	4	3	3	4.5	3.5	3

N37	5	3.5	2	4	4	0
N38	6	3	2.5	4	3.5	0
N39	4	2	1.5	6	3	3
N40	4	2.5	2.5	4	4	0
N41	7	2.5	3	4	9	2
N42	4	2	2.5	4.5	3	0
N43	7	3	2.5	6.5	4.5	0
N44	4	3	3	4	3	0
N45	5	3	3.5	4	4	1
N46	4	2.5	3	3	7	2
N47	6	2	2	3.5	4	1
N48	4	3	2.5	3.5	2.5	0
N49	6	5.5	3.5	3.5	2.5	2
N50	5	2	2	4.5	2.5	0

Patient	Baseline Treatment Site Lingual REC	Baseline Average Adjacent Site REC	Baseline Average Direct B&L Treatment Tooth REC	Baseline Average Direct B&L Adjacent Tooth REC	Baseline Average Treatment Tooth Opposite REC	Baseline Average Adjacent Tooth Opposite REC
N1	2	2.5	2.5	5	1	1.5
N2	0	0	1	0.5	0	0
N3	0	0	0	0	1.5	0
N4	3	0	3	2	2	0
N5	0	0.5	0	1.5	0	0
N6	0	0	0.5	0.5	0	0
N7	0	0	0	0	0	0
N8	0	0.5	2.5	0.5	1	0
N9	0	1.5	0.5	0	0.5	0
N10	0	0	0	0.5	0	0
N11	0	0	0	1	0	0
N12	2	1	2.5	1	1.5	0.5
N13	0	0.5	1.5	1	1.5	0
N14	0	0	0	0	0	0
N15	0	0	0	0	0	0
N16	3	2	2.5	4.5	2	4
N17	2	2	2	3	1	2.5
N18	0	0.5	1	0	0	0
N19	0	0	0.5	0	0	0
N20	2	1.5	1.5	1	0.5	0
N21	2	2.5	3	5	3.5	6
N22	0	1	0.5	0.5	0	0
N23	0	0	1	0	2.5	0
N24	0	0	1.5	2	0	0
N25	1	1.5	0.5	1	0	1
N26	0	0	1	0	0	0
N27	2	0	2.5	2.5	0.5	1.5
N28	1	0	1	1	0	0.5
N29	0	0	1.5	1.5	2	1
N30	1	0	3	0.5	2	0
N31	0	0	0	0.5	0	0.5
N32	5	3	3.5	3	2..5	2.5
N33	0	0	0.5	1.5	0	0
N34	1	0.5	0.5	0	0	0

N35	0	0	1	0	0.5	0
N36	2	3	1	5	2	3
N37	0	0	1.5	0	0	0
N38	0	0	0.5	0	0	0
N39	3	2.5	4	2.5	4	1.5
N40	0	0	0	1	0	0
N41	0	0	2	0	0.5	0
N42	0	0	0.5	0.5	0	0
N43	0	0	2.5	0	0	0
N44	0	0	0.5	0	0	0
N45	1	0.5	1.5	0	0.5	0
N46	0	2.5	1.5	1.5	1	1
N47	0	0	0.5	1	0	1
N48	0	0	0.5	0	0	0
N49	0	0	0.5	1	0	0.5
N50	0	0	0	0.5	0	0

Patient	Baseline Treatment Site Buccal PLAQUE	Baseline Treatment Site Lingual PLAQUE	Baseline Treatment Site Bone Height	Baseline Adjacent Site Bone Height	Number Teeth Baseline	Plaque index Baseline
N1	1	1	5.4	8.09	18	0.75
N2	0	0	4.58	4.41	26	0
N3	1	1	4.53	2.48	23	0.83
N4	0	1	4.34	1.38	26	0.75
N5	1	0	3.15	3.11	28	0.916
N6	0	0	7.83	5.17	19	0.167
N7	1	1	6.45	3.56	23	1
N8	1	1	4.67	4.66	26	0.83
N9	1	0	6.09	6.59	26	0.416
N10	1	1	4.31	2.86	26	0.5
N11	1	1	8.46	7.6	26	0.75
N12	0	1	6.28	3.83	20	0.416
N13	1	1	1.22	3.35	22	1
N14	0	1	5.59	5.73	27	0.33
N15	1	1	6.34	4.91	18	0.83
N16	1	1	7.37	8.9	27	0.916
N17	1	1	8.11	7.34	24	0.916
N18	1	1	5.94	6.2	28	0.58
N19	1	1	4.96	4.41	25	0.5
N20	1	1	6.94	6.91	21	1
N21	0	0	5.84	8.61	14	0
N22	1	1	3.47	3.57	12	0.416
N23	0	1	4.47	3.1	27	0.33
N24	0	0	7.87	8.62	22	0.167
N25	0	1	3.19	1.93	22	0.5
N26	0	1	5.44	3.19	25	0.58
N27	1	1	8.6	5.61	25	1
N28	0	1	4.6	4.54	25	0.5
N29	0	0	4.42	4.48	25	0.167
N30	1	0	3.94	3.53	25	0.58
N31	0	1	2.86	3.92	24	0.5
N32	0	1	3.83	7.25	23	0.5
N33	1	1	6.17	5.82	28	0.58
N34	1	1	8.75	5.01	25	0.83
N35	1	1	4.72	3.11	28	0.58
N36	1	1	4.6	6.04	25	0.916

N37	1	1	3.23	2.83	32	1
N38	1	1	2.2	3.64	32	1
N39	0	1	4.38	6.36	28	0.416
N40	1	1	1.93	2.96	25	1
N41	0	1	5.7	6.05	29	0.67
N42	1	1	3.85	2.47	29	1
N43	1	1	5.97	1.67	23	1
N44	1	1	3.26	2.94	20	1
N45	1	1	6.5	4.12	23	1
N46	1	1	5.61	4.17	12	1
N47	1	1	3.15	3.5	26	0.67
N48	1	1	5.91	7.31	24	0.83
N49	1	1	3.23	3.3	27	0.83
N50	1	1	3.94	1.85	25	0.75
	0 - no plaque	0 - no plaque				
	1 - plaque	1 - plaque				

Patient	12 Month Treatment Site Buccal PD	12 Month Treatment Site Lingual PD	12 Month Adjacent Site AVG PD	12 Month Treatment Tooth B&L AVG PD	12 month Adjacent Tooth B&L AVG PD	12 month Treatment Tooth Opposite AVG PD
N1	5	5	3.5	2.5	2.5	3.5
N2	4	5	3.5	1.5	1.5	3
N3	5	4	3	3	2.5	3
N4	4	4	3.5	2.5	2	3
N5	4	4	2.5	2	1.5	3
N6	3	3	3.5	3	2.5	3
N7	4	3	3	2	2	3.5
N8	5	5	3.5	2.5	2.5	3.5
N9	5	7	5	2.5	3.5	3
N10	4	5	4	3	3	4.5
N11	4	5	3	2	2	3
N12	6	6	3	2	2	3
N13	3	3	3	2	2	2
N14	4	5	3.5	2.5	2	4.5
N15						
N16	5	5	6	2	4	2.5
N17	3	3	4	3	2	3
N18	3	4	3.5	2	2	3
N19	3	3	4	3	2	3.5
N20	4	5	3	2	2	3
N21	4	3	3	2	1.5	3
N22	4	6	6	2.5	2.5	2.5
N23	3	4	2.5	2	2	2.5
N24	5	6	4.5	2.5	2	4
N25	4	5	4	3.5	2	3
N26	4	6	3.5	2.5	2.5	4
N27	3	3	2	1	1	2
N28	3	4	3	2.5	2.5	3.5
N29	3	3	3	2	2.5	4
N30	5	4	3	2.5	2.5	4.5
N31	5	5	5	4	2.5	3.5
N32	3	4	3	3.5	2	2.5
N33	3	6	5	3	3	5
N34	3	3	4	2	2	3
N35	5	5	4	2	2	5
N36	3	4	2.5	2.5	1.5	3.5

N37	3	5	3.5	2.5	2.5	3
N38	5	5	4.5	2.5	2.5	4
N39	3	5	3	2	2.5	3
N40	3	5	2	2	2	3
N41	4	4	4.5	2	2.5	2.5
N42	4	4	4	2	2.5	4
N43	4	4	4	2	2	3.5
N44						
N45	5	4	4	3	3	3.5
N46	3	4	3.5	2	2	2.5
N47	4	4	4.5	2	2.5	3
N48	5	5	3	2.5	2	3
N49	3	5	4	3.5	2.5	3
N50	3	3	3	2.5	2	4

Patient	12 month Adjacent Tooth Opposite AVG PD	12 Month Treatment Site Buccal Rec	12 Month Treatment Site Lingual Rec	12 Month Adjacent Site AVG REC	12 Month Treatment Tooth B&L AVG REC	12 Month Adjacent Tooth B&L AVG REC
N1	4.5	2	2	3	1.5	3.5
N2	2.5	0	0	0	0.5	0
N3	3	1	0	0	0.5	0
N4	3.5	2	2	0	2.5	1.5
N5	2	2	0	1.5	0.5	1.5
N6	3.5	0	0	1	1	1
N7	3.5	0	0	0	0	0
N8	2.5	0	0	0	2	0
N9	3.5	1	0	0.5	0	0
N10	5	1	0	0.5	0	0.5
N11	3	0	0	0	0	0.5
N12	3.5	2	3	2	2	1
N13	2.5	0	0	0	1	0
N14	3	0	0	0	0.5	0
N15						
N16	5	3	5	2..5	3	5
N17	4	1	1	0.5	1	2.5
N18	2	0	0	1	0.5	0
N19	4	1	0	0.5	1	0.5
N20	2.5	3	2	2	2.5	1
N21	2.5	3	2	3	3.5	5
N22	3.5	0	0	0	1	0.5
N23	2.5	0	1	0.5	2	1
N24	5.5	0	0	1.5	1	1.5
N25	2.5	0	1	0.5	0.5	1
N26	3.5	2	1	0	1	0
N27	3	4	2	0.5	3	3
N28	3	2	2	0	1	0
N29	3.5	1	0	1	1	1.5
N30	3.5	2	1	0	2	0
N31	3.5	0	0	0.5	0.5	1.5
N32	3.5	3	6	3	3.5	3
N33	3.5	0	0	1	0	2
N34	3.5	0	0	0.5	0.5	0
N35	3.5	0	0	0	1	0
N36	2.5	2	3	3.5	2.5	4.5

N37	3.5	0	0	0	0.5	0
N38	3.5	0	0	0	0.5	0.5
N39	3.5	2	2	2	2.5	2.5
N40	3	1	0	0.5	0.5	1.5
N41	6	2	2	1	2	0.5
N42	3.5	1	0	0	0.5	0.5
N43	3	3	3	1	4	2.5
N44						
N45	3.5	0	1	0	0.5	0
N46	3	1	1	1	1.5	2
N47	6	1	0	0.5	0.5	0.5
N48	2.5	1	1	1	1	0
N49	3.5	0	0	0	0	0.5
N50	3	2	1	0.5	0.5	0.5

Patient	12 month Treatment Tooth Opposite AVG REC	12 Month Adjacent Tooth Opposite AVG REC	12 Month Treatment Site Buccal PLAQUE	12 Month Treatment Site Lingual PLAQUE	12 Month Treatment Site Bone Height	12 Month Adjacent Site Bone Height
N1	0	1	1	1	6.75	8.54
N2	0.5	0	1	1	4.24	3.03
N3	0.5	0	0	0	4.38	2.56
N4	1	0	0	0	5.45	2.1
N5	0	0	0	0	3.25	3.11
N6	0	0	1	0	6.83	6.58
N7	0	0	0	1	5.26	4.45
N8	0.5	0	1	1	4.56	4.04
N9	0.5	0	1	1	6.44	6.12
N10	0	0	1	1	4.2	2.68
N11	0	0	1	0	7.33	6.49
N12	2	1	1	1	6.76	4.48
N13	0.5	0	0	1	1.88	3.09
N14	0	0	0	0	5.75	5.61
N15						
N16	2	1.5	1	1	7.43	8.51
N17	0	1.5	1	1	6.27	6.4
N18	0	0	1	1	4.4	4.56
N19	0	0	1	1	5.04	4.62
N20	0.5	0	0	0	4.5	6.22
N21	2.5	3	0	0	5.08	8.57
N22	0	0	0	1	4.44	3.96
N23	2.5	0	0	0	4.68	3.47
N24	0	0	1	1	6.34	8.63
N25	1	0.5	0	1	4.41	2.37
N26	0	0	1	1	5.14	4.04
N27	1.5	3	0	0	7.7	5.14
N28	0	0	1	1	3.8	3.58
N29	0.5	1	0	1	4.14	4.4
N30	1	0	1	1	4.27	4.1
N31	0	1	0	0	2.58	3.13
N32	2	2.5	1	1	3.34	6.57
N33	0	0	1	1	6.04	5.99
N34	0	0	0	0	4.46	3.39
N35	0	0	0	0	5.55	3.18

N36	2	2.5	1	1	5.2	6.87
N37	0	0	0	1	1.97	1.92
N38	0	0	1	1	3.21	3.67
N39	4	0.5	1	1	6.07	6.4
N40	0	2	0	0	2.11	2.81
N41	1	0	0	0	4.77	5.72
N42	0	1.5	1	1	3.76	2.92
N43	3	1	1	1	6.07	2.78
N44						
N45	0	0	1	1	6.67	5.13
N46	0	2	1	1	5.48	3.06
N47	0	0	1	1	2.63	1.65
N48	0.5	0	1	1	5.55	7.81
N49	0	0	0	1	2.48	2.35
N50	0	0	0	0	3.35	3.1
			0 - no plaque	0 - no plaque		
			1 - plaque	1 - plaque		

Patient	Number Teeth 12mo	Plaque index 12mo	Calibration Baseline Bone Height Treatment Site	Calibration Baseline Bone Height Adjacent Site	Calibration 12 Month Bone Height Treatment Site	Calibration 12 month bone height adjacent site
N1	18	0.67	5.59	8.22	6.58	8.46
N2	26	0.42	4.21	4.02	4.54	2.81
N3	23	0.167	3.69	3.9	3.85	2.39
N4	26	0.18	4.59	1.33	5.27	2.14
N5	28	0.33	3.16	3.01	3.33	3.2
N6	19	0.5				
N7	23	0.5				
N8	26	0.58				
N9	25	0.67				
N10	24	0.58				
N11	26	0.33				
N12	20	0.75				
N13	21	0.5				
N14	27	0				
N15						
N16	27	0.75				
N17	24	0.67				
N18	28	0.58				
N19	25	0.5				
N20	21	0.25				
N21	14	0				
N22	12	0.167				
N23	27	0.33				
N24	22	0.75				
N25	21	0.416				
N26	25	0.58				
N27	25	0				
N28	25	0.67				
N29	25	0.25				
N30	25	0.67				
N31	22	0.67				
N32	22	0.58				
N33	28	1				
N34	25	0.416				
N35	28	0.083				

N36	25	0.67				
N37	32	0.416				
N38	32	0.83				
N39	27	0.58				
N40	25	0.416				
N41	29	0.25				
N42	29	0.83				
N43	23	0.83				
N44						
N45	23	1				
N46	12	0.83				
N47	26	0.67				
N48	24	0.25				
N49	27	0.5				
N50	25	0.33				